REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

Claims 1 and 2 have been amended to recite that the crystals of "(S)-2-[[[3-methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole" are substantially free of crystals of the (R) isomer. Support is found in the specification, for example on pages 24-25 of the specification, wherein the crystals obtained have the optical purity of 99.4% ee.

Turning to the Official Action, claims 1, 2 and 4 are rejected under 35 USC 102 as anticipated by Barberich et al. (U.S. 2001/0025107), Nohara et al. (U.S. 4,628,098) and Kato et al. (U.S. 6,002,011). This ground of rejection is respectfully traversed as applied to the claims after the foregoing amendments.

The cited references fail to disclose crystals of (S)-2-[[[3-methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole which are substantially free of crystals of (R)-2-[[[3-methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole.

Accordingly, the cited references fail to anticipate the subject matter of claims 1, 2 and 4 under 35 USC 102.

Claims 1, 2 and 4 are rejected under 35 USC 103 as unpatentable over the combined teachings of Barberich et al., Nohara et al. and Kato et al., in view Chemical and Engineering News, U.S. Pharmacopia and Concise Encyclopedia Chemistry. This ground of rejection is also respectfully traversed.

As mentioned previously, the crystals of optically active compound ((S)-lansoprazole) of the present invention are completely different from known crystals of racemic lansoprazole (disclosed in USP 4,628,098 and USP 6,002,011). That is, the properties of crystals of (S)-lansoprazole are completely different from those of known crystals of racemic lansoprazole.

In this respect, there is submitted herewith a copy of KIKAN KAGAKU SOSETSU No. 6, 1989, pp. 32-33 (Reference Document 4) together with its English translation.

Reference Document 4 clearly shows that the physicochemical properties of crystals of an optically active compound such as a melting point, solubility, etc. are completely different from those of crystals of a corresponding racemate.

Properties of (S)-lansoprazole are actually quite different from those of racemic lansoprazole as shown in the attached Appendix I (X-ray powder diffraction has been set forth previously). In view of the melting points, X-ray powder diffraction patterns, etc. shown in Appendix I, racemic lansoprazole is considered to be a racemic compound rather than a racemic mixture, and on page 33, [Solubility], Reference Document 4 discloses as follows:

"since crystalline structure of crystals of a racemate in the form of a racemic compound or a racemic solid solution is essentially different from that of crystals of the optically active compound, there is no constant relation between the solubility of crystals of such racemate and that of the optically active compound, and the solubility may be higher in some cases or lower in other cases."

That is, the crystalline structure of (S)-lansoprazole is essentially different from that of racemic lansoprazole and the solubility of (S)-lansoprazole cannot be predicted from that of racemic lansoprazole. These facts clearly show that (S)-lansoprazole cannot be crystallized by the same method as crystallization of racemic lansoprazole. Further, since crystalline forms of (S)-lansoprazole and racemic lansoprazole are different from each other, it can be said that, as a chemical compound, (S)-lansoprazole is completely different from racemic lansoprazole.

Although U.S. 2001/0025107 discloses (S)-lansoprazole, there is no teaching or suggestion about "crystalline" (S)-lansoprazole in this reference.

Further, as the production process of (S)-lansoprazole, this reference refers to only WO 96/02535, WO 96/17077 and WO 97/02261 (See page 2, [0013] of U.S. 2001/0025107). WO 96/02535 and WO 97/02261 correspond to Reference Documents 2 and 3, respectively, and merely disclose the production of (S)-lansoprazole in the form of an oil. No crystals of (S)-lansoprazole are obtained in these documents (See Example 11 of Reference Document 2 and Example 21 of Reference Document 3).

More specifically, as mentioned previously, the Applicant of Reference Documents 2 and 3, i.e., ASTRA AKTEBOLAG, has studied the production and purification methods of benzimidazole proton pump inhibitor (PPI) compounds inclusive of lansoprazole for a long time. In spite of such studies, as to optically active lansoprazole, only the compound in the form of an oil has been obtained as shown by Reference Documents 2 and 3. Thus, from Reference Documents 2 and 3, a person skilled in the art would presume that optically active lansoprazole, in particular, (S)-lansoprazole in the form of crystals, cannot be obtained.

In fact, as seen from Example 11 of Reference Document 2, in the case of lansoprazole, racemic lansoprazole precipitates as crystals more easily in comparison with the optically active isomer and the mother liquor has a higher optical purity. This suggests that, even if racemic lansoprazole can be crystallized, its optically active isomer can be hardly crystalized.

Further, in Reference Document 2, the purification requires precipitation of the crystals of the racemic compound, concentration of the remaining mother liquor to improve the optical purity of the mother liquor, and then repetition of these procedures. Even if these procedures are repeated, crystals of the optically active isomer cannot be obtained and the isomer is obtained as an oil.

That is, Reference Documents 2 and 3 show that, in the case of lansoprazole, i) racemic lansoprazole precipitates as crystals more easily in comparison with the optically active isomer and the mother liquor has a higher optical purity, and ii) crystals of the optically active isomer cannot be obtained even if concentration of the mother liquor is repeated. This certainly suggests the difficulty of the crystallization of (S)-lansoprazole.

Thus, even in the presence of an asymmetric center in lansoprazole, a method of resolution of an optically active isomer, and the presence of crystals of racemic compound have been known in the cited reference U.S. 4,628,098, U.S. 6,002,001 and the like, such prior art knowledge does facilitate crystallization of an optically active isomer. In other words, the prior art including Document 2 and 3 suggests that the crystallization of the optically active isomer of lansoprazole would be very difficult, while racemic lansoprazole can be crystallized. This would dissuade a

person skilled in the art from trying the crystallization of the optically active isomer of lansoprazole rather than motivate the artisan towards the present invention.

WO 96/17077 (submitted herewith as Reference Document 5) merely discloses the production of a single enantiomer by stereoselective bioreduction and working examples disclosed are elution by chiral HPLC. Thus, Reference Document 5 is irrelevant to crystallization of lansoprazole.

Also, the Examiner rejects the patentability of the present invention as being obvious by citing (i) Chemical & Engineering News, (ii) U.S. Pharmacopia, (iii) Concise Encyclopedia Chemistry, as well as above-mentioned cited references. However, in the cited references (ii) and (iii), it is merely described generally that a crystal may exist in various forms and each one of the crystal forms has the characteristic lattice pattern in the X-ray powder diffraction. Therefore, these cited references are of no help at all to solve the problem of the present invention that is to obtain a crystal of optically active isomer of particular compound; lansoprazole. In addition, the description of the cited reference (i) is also generic. But since it was issued on February, 2003, its citation is respectfully submitted to be unavailable as prior art.

In view of the foregoing, it is respectfully submitted that the combined teachings of the cited references fail to disclose or suggest crystals of (S)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole which are substantially free of crystals of (R)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole. Moreover, the combined teachings of cited references would not have motivated one of ordinary skill in the art to prepare crystals of the (S) isomer. Further, it would have been unexpected to one of ordinary skill in the art to have obtained crystals of (S) isomer with ease according to the method of the present invention.

Claims 1, 2 and 4 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4 of USP 6,462,058.

A Terminal Disclaimer in compliance with PTO practice was submitted with the Applicants' last Response dated March 22, 2004. A copy of the Terminal Disclaimer is enclosed.

Accordingly, this ground of rejection is deemed to be overcome.

Lastly, claims 1, 2 and 4 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 and 10 of USP 4,628,098 in view of Barberich et al., Chemical and Chemical and Engineering News, U.S. Pharmacopia and Concise Encyclopedia Chemistry. This ground of rejection is respectfully traversed.

For the reasons described above, the combined teachings of the cited references fail to disclose or suggest crystals of (S)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole which are substantially free of crystals of (R)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole.

Thus, it is respectfully submitted that this ground of rejection is untenable and should be withdrawn.

In view of the foregoing, it is believed that each ground of rejection set forth in the Official Action have been overcome, and that the application is now in condition for allowance.

Respectfully submitted,

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Appendix I

Comparison of Properties of Racemic and (S)-Lansoprazole

	Racemic Lansoprazole	(S)-Lansoprazole
Appearance	crystalline white powder	crystalline white powder
Hygroscopicity	non	non
Polymorphism	2 kinds; developed with stable form (inclusive solvate) m.p. 166°C	m.p. of stable form (anhydride) 145°C
Solubility (25°C, mg/mL) pH 1 pH 5 pH 7 pH 9	lower than enantiomer 13 (dec.) <0.05 0.03 0.08	tend to be soluble in alkaline solution >30 (dec.) 0.032 0.13 0.27
Stability in solution (37°C) 1st fluid (pH 1.2) 2nd fluid (pH 6.8	residual ratio halved within about 2 min. 2h: 80%	residual ratio halved within about 2 min. 2h: 80%
Stability in solid state	relatively stable	somewhat unstable residual ratio 60°C 1 week : 96.4% 2 weeks: 93.9% 4 weeks: 87.4%
X-ray powder diffraction, peaks at interplanar spacings (Å)	15.22, 15.06, 6.18, 6.15, 5.20, 5.02, 4.72, 3.96, 3.85, 3.76, 3.55, 3.46, 3.19, 3.11, 2.93, 2.90, 2.85, 2.69, 2.66	11.68, 6.78, 5.84, 5.73, 4.43, 4.09, 3.94, 3.90, 3.69, 3.41, 3.11

English Translation of Reference Document 1

The New Scientists Group, edited by Yazawa Science Office, pages 5-9, published by Keiso Shobo, November 20, 1989 pages 5-9

RUPERT SHELDRAKE
The Hypothesis of Formative Causation
by HANAZUMI Yoko

1. A Theory of Morphogenetic Fields by Rupert Sheldrake
-Why is a rabbit not able to become an animal other than a rabbit?

· An "unseen field" linking glycerin in various places to each other

About 250 years ago, colorless liquid glycerin was extracted from a naturally occurring fat. After that, glycerin was widely used as a raw material of sweeteners, medicines, and nitroglycerin. However, this liquid had mysterious nature. In order to obtain crystals of glycerin, chemists tried every possible means such as rapid cooling, re-heating, etc., but, for some reason, this liquid was not crystallized at all. Then, finally, glycerin was considered not to be present in a solid state, while the reason was unknown.

Nevertheless, in the beginning of this century, a strange thing was happened. A manufacturer was going to send glycerin produced at a factory in Vienna, Austria to a customer in London by a cargo boat. Although the boat was caught in a heavy storm during the voyage, she arrived in London safely. Then, when the customer opened barrels of glycerin to investigate the contents, glycerin in one of the barrels was completely crystallized. The customer was upset because "such a thing was of no use to him". However, chemists were very happy. They tried to get first such glycerin crystals firstly formed in the world and applied themselves to crystallization of their own liquid glycerin using such crystals as seeds. Glycerin was crystallized in all over the world one after another, while it seemed incredible that, up to that time, no one succeeded in crystallization of glycerin for about 200 years. Further, when a chemist in a laboratory in U.S.A. got the same crystals and succeeded in crystallization of a sample, a remarkable incidence was happened. All glycerin in the laboratory began to be crystallized spontaneously. Among them, there was glycerin even placed in a sealed container! It was as if glycerin placed around the sample glycerin "learned" the process of crystallization of the sample glycerin and imitated the process all together.....

As seen from the above example of glycerin, in case of a newly found or synthesized chemical compound, in many cases, one often has a very hard time until a first crystal is obtained. That is, even if a solution of a compound is cooled, it is not easily solidified. However, once someone has succeeded in crystallization at any place in the world, since then, the crystallization can be readily carried out. As the number of crystallization increases, crystallization can be carried out more easily. - This "phenomenon" has been considered heretofore to be "infection" of a solution with debris of a crystal. The "infection" is taken place via a human being and also includes airborne infection wherein seeds are carried through the air and then fallen into a solution. If so, the reason why glycerin without seeding has been suddenly crystallized at the laboratory in U.S.A. can be elucidated. However, how about the reason why

glycerin without seeding has been crystallized in laboratories all over the world? This can also be explained by such a reason that debris of a crystal has been adhered to hairs or clothes of chemists coming and going between laboratories, or "airborne infection" has been taken place in a large scale, can't this? Realistically, it cannot be considered that so many chemists hustle around the world to "deliver seeds". So, there must be little likelihood that small debris of a crystal are carried across the Pacific Ocean or the Atlantic Ocean by the wind to enter into one room - a room of a laboratory where a glycerin sample is placed - among huge number of buildings. Even if there would be a certain possibility, an unanswerable question is still remained in this case of glycerin. It is the fact that glycerin is crystallized even in a sealed container which is insulated from the atmosphere in the room.

In addition, such crystals have another interesting point. That is, it is difficult to predict what crystalline form is taken by a chemical compound which is never crystallized, though such a prediction would not be impossible. One may consider that crystalline forms could be predicted based on its chemical composition, chemically, physically and experientially. Indeed, it would be possible. However, theoretically drawn up blueprints of a crystal amount to several ten kinds-no, to several hundred kinds, each probability of them is the same and law, and it cannot be determined beforehand (at least, it is impossible by the current science). And, no one knows which crystalline form is a correct one until the crystal is actually formed. Further, when a substance once "selects" its crystalline form, then, the substance continues to take that crystalline form. The current science however cannot answer to the first question on why a specific form is selected. Similarly, it cannot answer to a question on why the substance stays in the form of the first crystalline form.

There would be many people who wonder that the current science of the 20 century cannot yet clearly elucidate the mysterious phenomena relating to the above crystals arose in chemical fields. This is because, different from investigations of the origin of the universe and the origin of a substance wherein literally unseen or untouchable subjects are handled, crystals and crystallization per se are very popular and familiar phenomena. However, many of chemists take such an optimistic view that everything will be elucidated soon. They believe that, someday, all of unknown mechanisms of crystallization will be found out and, "inevitably", all events will be proved. Further, regarding the phenomena of sudden appearance of a new crystal and its rapid spreading, they seem to be satisfied by the conventional explanation such as "infection" rather than to establish a convincing explanation instead. Perhaps, the crystallization of glycerin in a sealed container must be considered to be an error rather than an exceptional case, - e.g., the cap or container was opened -.

Nevertheless, a person who elucidates this question from a completely new viewpoint has appeared, a young biochemist, Rupert Sheldrake, came from U.K. He has jumped drastically from such a conventional mechanistic concept that the formation of crystals must be completely illustrated from the view points of chemical and physical interactions, and has proposed a concept of an unknown "unseen force" which causes "morphogenesis" of crystals. He says that, by assuming this unseen action, the mysterious phenomena shown by crystals can be much more easily illustrated without relying upon accidental factors such as "infection", etc. Further, he says that crystalline forms vary depending upon kinds of chemical substances because this unseen action limitedly acts

on the same kind of chemical substance, in other words, there is an "unseen field" which acts on the same kind of chemical substance. For instance, it can be said that the occurrence of the crystallization of glycerin in all over the world is not due to the infection with seeds of a crystal, but is due to the transmission of the crystallization process through an unseen field which links the same kind of substance, glycerin, to each other. The same reason is applicable to the crystallization of glycerin in a sealed container.

- Mechanism of morphogenesis field

Sheldrake calls this unseen filed as a "morphogenesis field", and considers that its action participates not only in crystals but also in all shaped or formed substances existing in the natural environment. For instance, the expression of inherent own morphology in an organism is due to a "morphogenesis field", e.g., a human being has his own morphogenesis field and a rat has its own morphogenesis field.....

English Translation of Reference Document 4

KIKAN KAGAKU SOSETSU No. 6, 1989, edited by Nippon Kagaku-Kai
Pages 32-33

3 Preferential Crystallization

Kazuhiko SAIGO

The preferential crystallization mentioned in this chapter is also called as selective crystallization or inoculation crystallization, and is characterized in that this method is essentially different from other methods of optical resolution because no asymmetric factor is required in this method. However, only a very few cases of optical resolution by preferential crystallization have been reported and only 248 cases are described in a literature edited by J. Jacques, the authority in this art field, et al. Even at present, only about 300 cases inclusive of subsequent reports are known. Such a small number of cases despite the superiority of preferential crystallization as a method of optical resolution are due to its applicability to only special crystals. This chapter summarizes the fundamental and examples of optical resolution by this preferential crystallization.

1. Crystalline morphology and properties of racemate

A racemate present in the form of gas, liquid or a solution state shows properties of a mixture of equal amounts of both enantiomers. However, properties of a racemate in a crystalline state vary depending upon a particular crystalline form. When some racemate is crystallized from a melting state or a solution state, the racemate takes one of the following three crystalline forms.

(1) Racemic mixture or conglomerate - each of a single crystal is composed of a single enantiomer, but both enantiomers are present in the ratio of 50 : 50 in the crystals of a racemate as a whole.

(2) Racemic compound - each of a single crystal is composed of a pair of both enantiomers, and thus the enantiomer ratio in a single crystal is 50 : 50.

(3) Racemic solid solution - each of a single crystal is composed of both enantiomers having various enantiomer ratio, but both enantiomers are present in the ratio of 50 : 50 in the crystals of a racemate as a whole.

Fig. 1 illustrates these crystalline forms, schematically.

Fig. 1

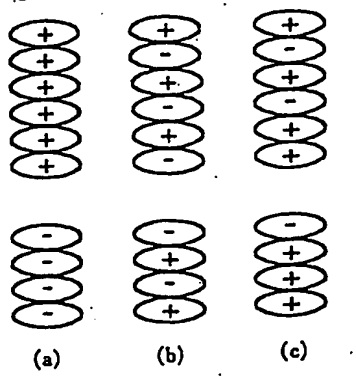

Fig. 1 Schematic view of three crystalline forms
(a) racemic mixture, (b) racemic compound, (c) racemic solid solution

When physicochemical properties of crystals of a racemate in these three crystalline forms are compared with physicochemical properties of crystals of its optically active compound, the following differences are present.

[Melting point] A melting point of crystals of a racemate in the form of a racemic mixture (using a sample prepared by grinding a certain number of single crystals very well) is lower than that of crystals of its optically active compound (Fig. 2 (a)). This is theoretically the same as the case just like a mixed examination of two kinds of compounds having the same melting points is conducted because crystals of a racemic mixture is a 1 : 1 mixture of crystals of both optically active compounds. On the other hand, in case of crystals a racemate in the form of a racemic compound, since its crystalline structure is clearly different from that of the optically active compound, there is either case that the melting point of the racemate is higher or lower than that of the optically active compound. However, in general, there are many cases that the melting point of the racemate is higher than that of the optically active compound (Fig. 2 (b)). The melting point of a racemic solid solution varies subtly depending upon a particular enantiomer ratio in the crystals, and has a feature different from those of a racemic mixture and a racemic compound (Fig. 2 (c)).

Fig. 2

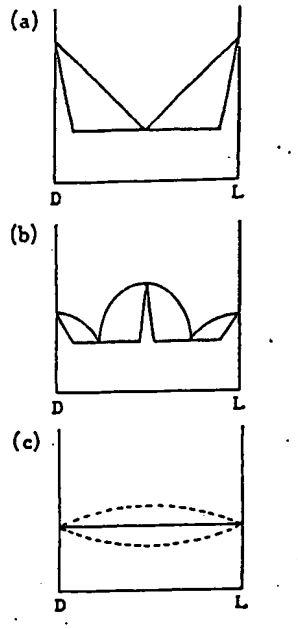

Fig. 2 Two-Component System Melting Pint Diagram
(a) racemic mixture, (b) racemic compound, (c) racemic solid solution

[Solubility] Since crystals of a racemate in the form of a racemic mixture are a mixture of equal amounts of crystals of both optically active compounds, its solubility corresponds to double the solubility of crystals of each optically active compound. In contrast, since crystalline structure of crystals of a racmate in the form of a racemic compound or a racemic solid solution is essentially different from that of crystals of the optically active compound, there is no constant relation between the solubility of crystals of such racemate and that of the optically active compound, and the solubility may be higher in some cases or lower in other cases.

[IR spectra, X-ray diffraction patterns] Crystals of a racemate in the form of a racemic mixture is a mixture of equal amounts of crystals of both optically active compounds. Further, both active compounds have an enantiomeric relationship, and respective crystals must therefore give the exactly same IR spectra and X-ray diffraction patterns. Then, in case of a racemic mixture, crystals of a racemate and crystals of its optically active compound gives the same IR spectra and X-ray diffraction patterns. On the other hand, in case of a racemic compound or a racemic solid solution, crystalline structure of crystals of a racemate and that of crystals of its optically active compound are essentially different from each other and their IR spectra and X-ray diffraction patterns are therefore different from each other. However, the differences between them are small and, in many cases, only subtle parts are different from each other.

As described above, there are three crystalline forms in crystals of a racemate and their physicochemical properties are different from each other. "The optical resolution by preferential crystallization of this chapter is applicable to only when crystals are those of a racemate in the form of a racemic mixture."

季刊 化学総説
No. 6, 1989
KIKAN KAGAKU SOSETSU

光学異性体の分離

日本化学会編

学会出版センター

3 優先晶出法

西 郷 和 彦

本章で述べる優先晶出法は，選択晶出法あるいは接種法ともいわれている方法であり，不斉要素をまったく必要としない点で，他の光学分割法と本質的に異なる特徴をもっている．しかし反面，優先晶出法による光学分割の例はきわめて少なく，この分野の権威者である J. Jacques らがまとめた成書[1]には，248 例が記載されているにすぎない．その後の報告も含めて，現在でも約 300 例ほどである．このように，優先晶出法が光学分割手法として優れているにもかかわらずその例が少ないのは，特殊な結晶の場合にのみ適用可能なためである．本章では，この優先晶出法による光学分割の基礎と実例について概述する．

1 ラセミ体の結晶形態と性質

気体，液体あるいは溶液状態として存在するラセミ体 (racemate) は，両エナンチオマーの等量混合物としての性質を示すが，結晶状態のラセミ体は結晶形態によって異なった性質を示す．あるラセミ体を溶融状態あるいは溶液状態から結晶化させると，ラセミ体は次の三つの結晶形態の一つをとる．

① ラセミ混合物 (racemic mixture あるいは conglomerate)——単結晶一つ一つは単一のエナンチオマーから成り立っているが，ラセミ体結晶全体では両エナンチオマー比が 50：50 になっている結晶．

① ラセミ化合物 (racemic compound)——一つ一つの単結晶が対をなした両エナンチオマーから成り立っており，したがって各単結晶中のエナンチオマー比が 50：50 になっている結晶．

③ ラセミ固溶体 (racemic solid solution)——一つ一つの単結晶に存在する両エナンチオマーの比が一定でないが，ラセミ体結晶全体のエナンチオマー比は 50：50 になっている結晶．

これらの結晶形態を模式的に示すと図 1 のようになる．

これら三つの結晶形態にあるラセミ体結晶の物理化学的性質を光学活性体結晶の物理化学的性質と比較すると，次のような差異がある．

[融　点] ラセミ混合物であるラセミ体結晶の融点（ある相当数の単結晶をよくすりつぶし調製した試料を用いる）は，光学活性体結晶の融点よりも低い（図 2(a)）．これは，ラセミ混合物

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3 Preferential Crystallization

Kazuhiko SAIGO（東京大学工学部）

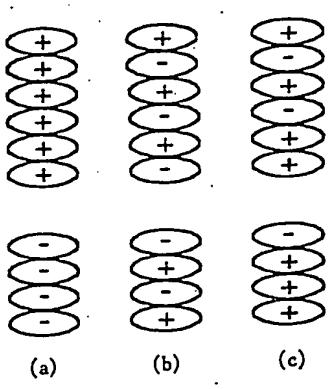

図 1　3種の結晶形態の模式図. (a)ラセミ混合物, (b)ラセミ化合物, (c)ラセミ固溶体.

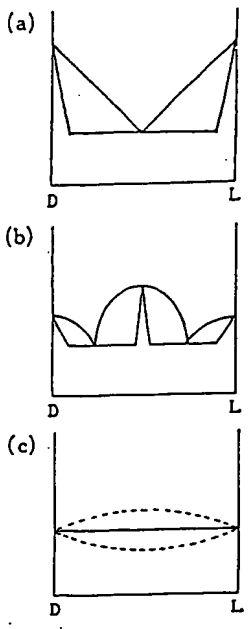

図 2　2成分系融点図. (a)ラセミ混合物, (b)ラセミ化合物, (c)ラセミ固溶体.

結晶が両光学活性体結晶の1：1混合物であり，ちょうど同じ融点をもつ2種類の化合物の混融試験をやった場合と同じ原理である．一方，ラセミ化合物であるラセミ体結晶の場合，その結晶構造が光学活性体のものとは明確に異なるので，融点は光学活性体結晶よりも高い場合も低い場合もある．しかし，一般には，ラセミ体結晶の融点が光学活性体の結晶の融点よりも高いことが多い（図 2(b)）．ラセミ固溶体結晶の融点は，結晶中のエナンチオマー比によって微妙に変化し，ラセミ混合物，ラセミ化合物とは異なった様子を示す（図 2(c)）．

[溶解度]　ラセミ混合物であるラセミ体結晶は両光学活性体結晶の等量混合物であるので，その溶解度はそれぞれの光学活性体結晶の溶解度の2倍になる．これに対し，ラセミ化合物，ラセミ固液体であるラセミ体結晶は光学活性体結晶と結晶構造が本質的に異なるため，ラセミ体結晶の溶解度は光学活性体結晶の溶解度と一定の関係になく，大きいことも小さいこともある．

[赤外スペクトル，X線回折図]　ラセミ混合物であるラセミ体結晶は，両光学活性体結晶の等量混合物である．また，両活性体はエナンチオマーの関係にあるので，それぞれの結晶はまったく同一の IR スペクトル，X線回折図を与えるはずである．したがって，ラセミ混合物の場合，ラセミ体結晶と光学活性体結晶は同一の IR スペクトル，X線回折図を与える．一方，ラセミ化合物，ラセミ固溶体の場合，ラセミ体結晶と光学活性体結晶の結晶構造は本質的に異なることから，それらの IR スペクトル，X線回折図は異なる．しかし，その差異は小さく，微妙なところで異なっているにすぎないことが多い．

このように，ラセミ体結晶には3種類の結晶形態があり，物理化学的性質を異にしている．本章で取上げる「優先晶出法による光学分割は，ラセミ体結晶がラセミ混合物のときにのみ適用可能」である．

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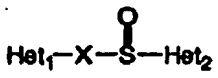
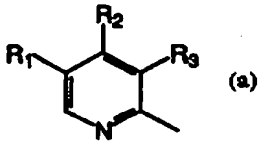
(54) Title: ENANTIOSELECTIVE PREPARATION OF PHARMACEUTICALLY ACTIVE SULFOXIDES BY BIOREDUCTION

(57) Abstract

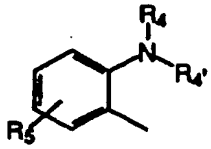
A compound of formula (II), either as a single enantiomer or in an enantiomerically enriched form, wherein Het_1 is (a) or (b), and Het_2 is (c) or (d), and is (e) or (f) (wherein N in the benzimidazole moiety of Het_2 means that one of the carbon atoms substituted by any one of R_6 to R_9 optionally may be exchanged for an unsubstituted nitrogen atom; R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy; R_4 and R_4' are the same or different and selected from hydrogen, alkyl, aralkyl; R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy; R_6-R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6-R_9 may complete together with the carbon atoms to which they are attached optionally substituted ring structures; R_{10} is hydrogen or alkoxycarbonyloxymethyl; R_{11} is hydrogen or forms an alkylene chain together with R_3; R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl) is obtained by stereoselective bioreduction of a compound of formula (II) in racemic form.



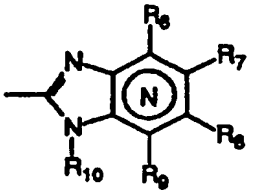
(II)



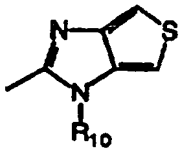
(a)



(b)

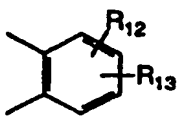


(c)



(d)

(e)



(f)

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Enantioselective preparation of pharmaceutically active sulfoxides by bioreduction

The present invention relates to a method of obtaining compounds as defined below, either as a single enantiomer or in an enantiomerically enriched form.

Background to the Invention

The racemic form of the compounds prepared by the method of the present invention are known compounds. Some of the compounds are also known in single enantiomeric form. The compounds are active H^+K^+ATPase inhibitors and they, including their pharmaceutically acceptable salts, are effective acid secretion inhibitors, and known for use as antiulcer agents. The compounds, which include the known compounds omeprazole (compound of formula (IIa) below), lansoprazole (compound of formula (IIc) below) and pantoprazole (compound of formula (IIb) below), are known for example from European Patent specifications EP 5129 and 124495, EP 174726 and EP 166287.

These compounds, being sulfoxides, have an asymmetric centre in the sulfur atom, i.e. exist as two optical isomers (enantiomers). It is desirable to obtain compounds with improved pharmacokinetic and metabolic properties which will give an improved therapeutic profile such as a lower degree of interindividual variation.

The separation of enantiomers of omeprazole in analytical scale is described in e.g. J. Chromatography, 532 (1990), 305-19. Also the separation of enantiomers of compounds with which the present invention is concerned, including omeprazole and pantoprazole, is described in German Patent Specification DE 4035455.

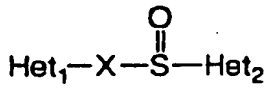
Recently there has been a great deal of literature published relating to the synthesis of optically active compounds using biocatalysts. The majority of this work has been aimed at finding routes to single enantiomer forms of pharmaceuticals. The reactions

receiving most attention have been those involved in the preparation of esters, acids and alcohols due to the general utility of these funtionalities in synthesis and also because the biocatalysts are readily available.

Studies on the synthesis of optically active sulfoxides are relatively rare partly due to the small number of pharmaceuticals containing sulfoxide groups and partly due to the fact that enzymes that react with the sulphur centre are not available commercially. The enantioselective reduction of methylphenylsulfoxide to the sulfide has been discussed by Abo M., Tachibana M., Okubo A. and Yamazaki S. (1994) Biosci. Biotech. Biochem. 58, 596-597.

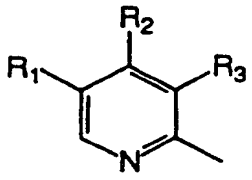
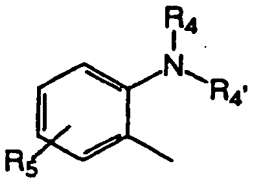
Description of the Invention

According to the present invention there is provided a method of obtaining a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

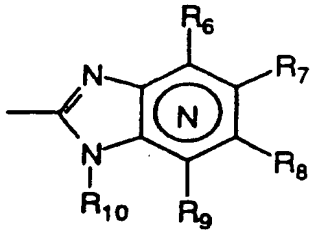
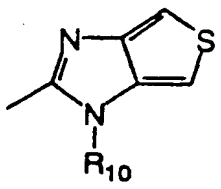


(II)

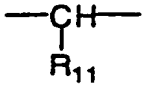
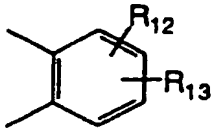
wherein:

Het_1 is  or 

and

Het $_2$ is  or 

and

X is  or 

wherein:

N in the benzimidazole moiety of Het_2 means that one of the carbon atoms substituted by any one of R_6 to R_9 optionally may be exchanged for an unsubstituted nitrogen atom;

R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylalkoxy;

R_4 and $R_{4'}$ are the same or different and selected from hydrogen, alkyl, aralkyl;

R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

R_6 - R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6 - R_9 may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

R_{10} is hydrogen or alkoxycarbonyloxymethyl;

R_{11} is hydrogen or forms an alkylene chain together with R_3;

R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl;

which method comprises stereoselective bioreduction of a compound of formula (II) in racemic form.

The compounds of formula (II) are active H^+K^+APTase inhibitors.

The compounds of formula (II) possess a stereogenic (asymmetric) centre which is the sulphur atom which forms the sulfoxide group between the Het_1-X- and Het_2 moieties. The compounds of formula (II) generally are a racemic mixture initially.

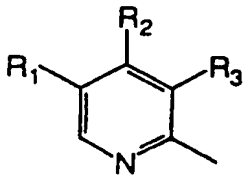
In the method according to the present invention the starting compound of formula (II) in racemic form is stereoselectively bioreduced to the corresponding sulfide of the formula:

$$Het_1—X—S—Het_2 \qquad (I)$$

(wherein Het_1, X and Het_2 are as defined above). Thus there is obtained compound of formula (II) as a single enantiomer or in enantiomerically enriched form which may be separated from the sulfide produced.

In the above definitions alkyl groups or moieties may be branched or straight chained or comprise cyclic alkyl groups, for example cycloalkylalkyl.

Preferably:

Het $_1$ is 

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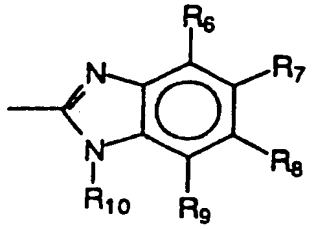
Het $_2$ is 

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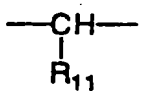
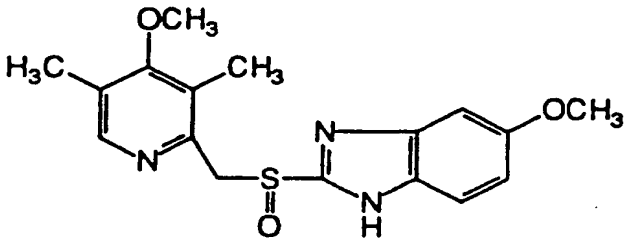
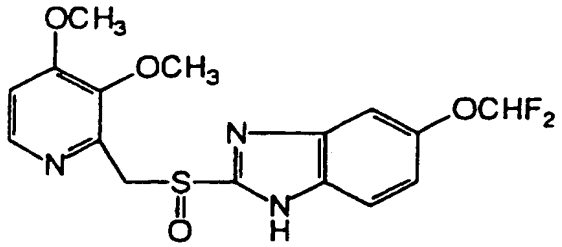
X is 

(wherein R_1, R_2, R_3, R_6 - R_9, R_{10} and R_{11} are as defined above).

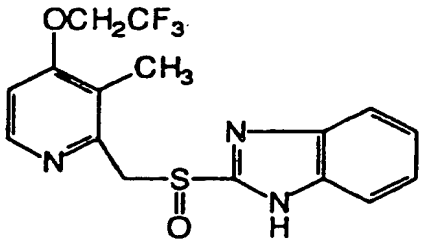
Most preferably the compounds with which the method of the present invention is concerned are compounds of the formula (IIa) to (IIe):



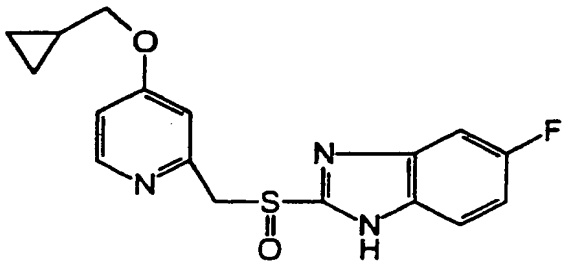
(IIa)



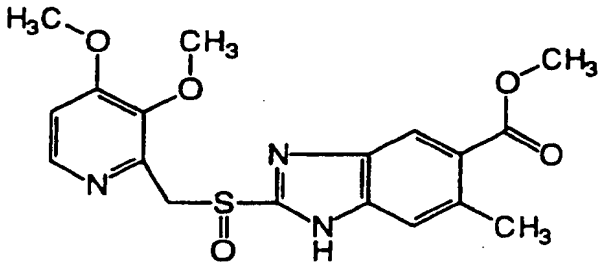
(IIb)



(IIc)

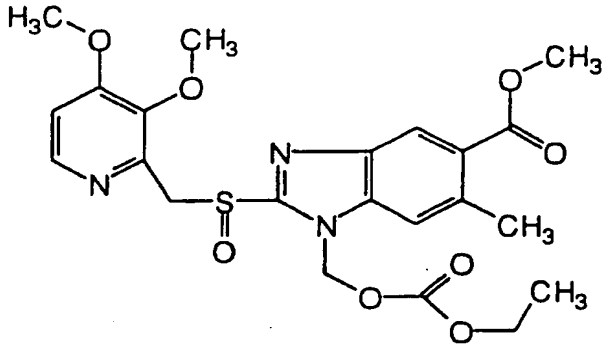


(IId)



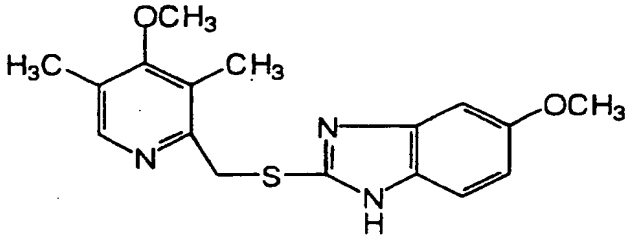
(IIe)

An example of a compound of formula (II) wherein R_{10} is alkoxycarbonyloxymethyl is

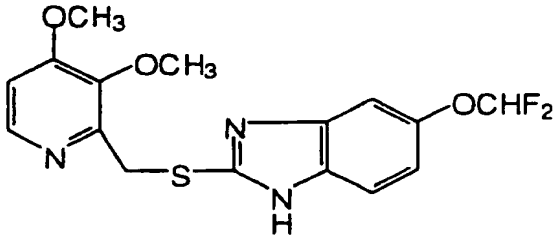


(IIf)

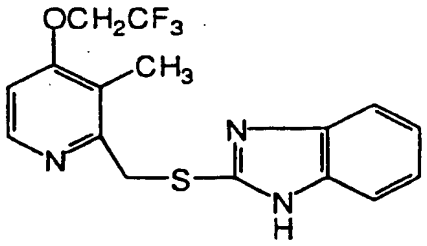
The sulfides formed from the compounds (IIa) - (IIf) will be respectively



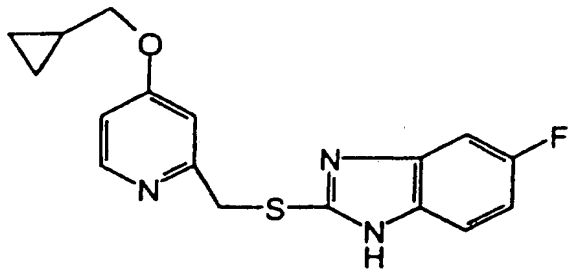
(Ia)



(Ib)



(Ic)



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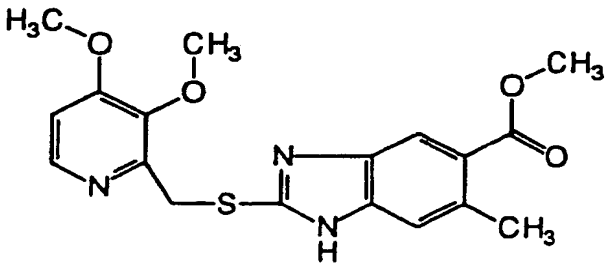
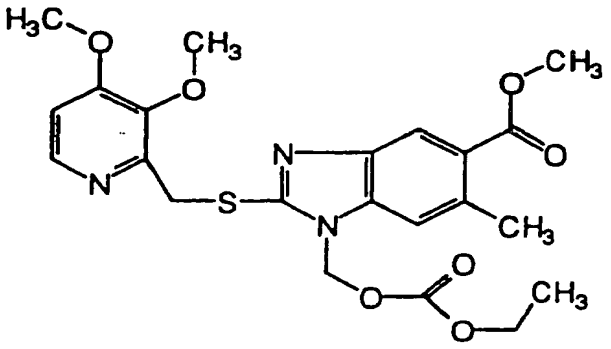


(Ie)



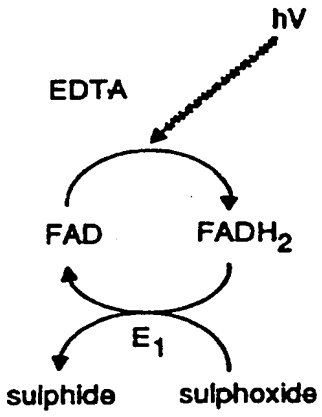
(If)

The stereoselective bioreduction according to the present invention may be carried out using a microorganism or an enzyme system derivable therefrom. The organisms used in the method according to the present invention are suitably organisms containing DMSO reductase, for example enterobacteriaceae such as E. Coli and Proteus sp., and purple non-sulfur bacteria of the genus Rhodobacter.

Also there may be used DMSO reductase to effect the bioreduction.

Using DMSO reductase, under anaerobic conditions a cofactor flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) for example may be photo-reduced in the presence of ethylene diamine tetraacetic acid (EDTA). The re-oxidation of the FAD or FMN is coupled to the reduction of the sulfoxide via the DMSO reductase. Hence an artificial biocatalytic cycle is generated in the absence of the cells natural anaerobic electron transport mechanism.

Photo catalysed DMSO Reductase Reaction



E_1 - DMSO reductase

The compounds of formula (II) are generally acid labile and thus the use of acid conditions is to be avoided. Generally the method according to the invention may be carried out at a pH of 7.6 to 8, suitably about 7.6, and at temperature of 25 to 35°C, suitably about 28°C.

According to one embodiment of the invention the method comprises contacting the compound of formula (II) with a microorganism which is:

Proteus vulgaris
Proteus mirabilis
Escherichia coli
Rhodobacter capsulatus

The microorganisms used are preferably:

Proteus vulgaris NCIMB 67
Proteus mirabilis NCIMB 8268
Escherichia coli ATCC 33694
Rhodobacter capsulatus DSM 938

These microorganisms are available from the following culture collections:

NCIMB
National Collection Of Industrial And Marine Bacteria
23 Saint Machar Drive
Aberdeen AB2 1RY
United Kingdom

ATCC
American Type Culture Collection
12301 Parklawn Drive
Rockville Maryland 20852
United States of America

DSM
Deutsche Sammlung von Mikroorganismen
Mascheroder Weg 1b
D-38124
Braunschweig
Germany

The present invention will now be illustrated with reference to the Examples.

EXAMPLE 1

The reductive resolution of the compound of formula (IIa) was investigated using whole cells of E. Coli ATCC 33694, Proteus vulgaris NCIMB 67, Proteus mirabilis NCIMB 8268 and a preparation of DMSO reductase from Rhodobacter Capsulatus DSM 938. E. Coli and the two strains of Proteus were grown under essentially anaerobic conditions in 1 litre screw capped flasks containing 800 ml of medium at 35°C for 48 hours on a rotary shaker. The basal culture medium used had the following composition (g/l): NaH_2PO_4, 1.56; K_2HPO_4, 1.9; $(NH_4)_2SO_4$, 1.8; $MgSO_4 \cdot 7H_2O$, 0.2; $FeCl_3$, 0.005; $Na_2MoO_4 \cdot 2H_2O$, 0.001; casamino acids, 1.5. After autoclaving this was supplemented with the following components (g/l): glycerol (for Escherichia coli) or glucose (for Proteus mirabilis), 5; thiamine·HCl, 0.03; nicotinic acid, 0.007. Dimethylsulfoxide (70mM) was added to the medium to serve as terminal electron acceptor for anaerobic respiration and as inducer for dimethylsulfoxide (DMSO) reductase. Trace elements solution (1 ml/litre) was also added to the medium. The stock trace elements solution contained (g/l): $CuSO_4 \cdot 5H_2O$, 0.02; $MnSO_4 \cdot 4H_2O$, 0.1; $ZnSO_4 \cdot 7H_2O$, 0.1; $CaCO_3$, 1.8. E. Coli was also grown anaerobically on the same medium under the same conditions but with 40 mM of fumarate as electron acceptor.

Cells were harvested by centrifuging and washed twice in 10 mM phosphate buffer, pH 7.6. The cells were then resuspended in 100 mM phosphate buffer, pH 7.6 to give a dry cell weight concentration of 3-6 g/l.

50 ml of each cell suspension was then placed in an autotitrator vessel and stirred without aeration in the presence of 0.1-0.3 g/l substrate and glucose (2.5%) at 35°C. The pH was maintained at 7.6 by autotitration with 0.5 mM NaOH.

Cells of Rhodobacter capsulatus DSM 938 where grown and DMSO reductase enzyme prepared as describes in Example 2, below. The reductive resolution of the compound

of formula (IIa) using said enzyme preparation was then carried out as describes in Example 2, except that the compound of formula (IIa) was present at a substrate concentration of 2.9 mM.

Detection of Products

The bioreduction of the compound of formula (IIa) was followed by reverse phase HPLC on a Spherisorb S5-ODS2 reverse phase column eluted with a 50:50 mixture of acetonitrile and 25 mM sodium phosphate buffer, pH 7.6 at a flow rate of 0.8 ml/min. Under such conditions the compounds of formula (IIa) and (Ia) were well resolved with retention times of 5.2 and 9.8 minutes respectively. Both compounds were detected at a wavelength of 300 nm.

The enantiomeric composition of the compound of formula (IIa) remaining was investigated by the following method. After removal of biomass the aqueous media was extracted with two volumes of ammonia saturated dichloromethane. The pooled organic extracts were dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to afford a pale brown solid. Then the enantiomeric composition of sulfoxide was determined by HPLC on a Chiralpak AD Column under the following conditions:

Column	Chiralpack AD 250 mm x 4.6 mm interior diameter with 50 mm guard column
Eluent	Hexane:Ethanol:Methanol (40:55:5% V/V)
Flow	1.0 ml/min
Injection Volume	20 µl
Wavelength	300 nm
Retention times	
Compound of formula (Ia)	5.1 min

Compound of formula (IIa):

(+) Enantiomer 8.5 min

(-) Enantiomer 13.4 min

The results in Table 1 were obtained for E. Coli ATCC 33694:

TABLE 1

Electron Acceptor	Dry Cell Weight g/l	Time (min)	Compound of formula (IIa) (ppm or mg L^{-1})	Compound of formula (Ia) (ppm or mg L^{-1})	ee % (+)	E
Fumarate	6	0	76	0	-	-
		5	65	10	-	-
		15	42	33	-	-
		30	33	42	-	-
		40	30	45	-	-
		65	27	47	-	-
		90	25	50	-	-
		130	22	53	>99	11
DMSO	6	0	74	0	0	-
		15	47	22	28	-
		35	40	34	74	-
		45	34	38	90	20

In the above Table E is the enantiospecificity constant which may be determined from the extent of conversion and enantiomeric excess of the unreacted compound from the following equation:

$$E = \frac{\ln[(1 - C) \times (1 - ee_s)]}{\ln[(1 - C) \times (1 + ee_s)]}$$

where C = conversion

ee_s = enantiomeric excess of the unreacted compound.

Also in the above table ee is the enantiomeric excess value for the (+) enantiomer of the compound of formula (IIa). The enantiomeric excess value gives an indication of the relative amounts of each enantiomer obtained. The value is the difference between the relative percentages for the two enantiomers. Thus, for example, when the percentage of the (-) enantiomer of the remaining sulphoxide is 97.5% and the percentage for the (+) enantiomer is 2.5%, the enantiomeric excess for the (-) enantiomer is 95%.

At a starting concentration of 0.3 g/l both P. vulgaris NCIMB 67 and P. mirabilis NCIMB 8268 afforded the (+) enantiomer of the compound of formula (IIa) in >99% enantiomeric excess after 48 h (Table 2) in yields of 32% and 5% respectively.

TABLE 2

	P. vulgaris NCIMB 67		P. mirabilis NCIMB 8268	
	Ratio of Enantiomers		Ratio of Enantiomers	
	(+)	(-)	(+)	(-)
Time hours				
0	50	50	50	50
24	79	21	67	33
48	>99	<1	>99	<1

The final concentration of the compound of formula (IIa) after 48 hours was 48 mg/litre for P. vulgaris NCIMB 67 and 8 mg/litre for P. mirabilis NCIMB 8268.

At a lower starting concentration of 0.1 g/l both organisms again afforded the (+) enantiomer of the compound of formula (IIa) in >99% enantiomeric excess, but after 24 hr (Table 3A) in 44% and 40% yields for P. vulgaris NCIMB 67 and P. mirabilis NCIMB 8268 respectively.

TABLE 3A

	P. vulgaris NCIMB 67		P. mirabilis NCIMB 8268	
	Ratio of Enantiomers		Ratio of Enantiomers	
	(+)	(-)	(+)	(-)
Time hours				
0	50	50	50	50
5	78	22	79	21
24	>99	<1	>99	<1

The final concentration of the compound for formula (IIa) after 24 hours was 22 mg/litre of P. vulgaris NCIMB 67 and 20 mg/litre for P. mirabilis NCIMB 8268.

The compound of formula (IIa) acts as a substrate for the isolated DMSO reductase from Rhodobacter capsulatus as shown in Table 3B. The conversion of total sulfoxide after 15 minutes and after 1 hour was 80% and > 90% respectively.

TABLE 3B

	R. capsulatus DSM 938	
	Ratio of Enantiomers	
	(+)	(-)
Time		
0	50	50
15 minutes	50	50
1 h	15	85

EXAMPLE 2

The reductive resolution of compounds of formula (IIb) and (IIc) was investigated with E. coli ATCC 33694, P. vulgaris NCIMB 67, and a preparation of DMSO reductase from R. capsulatus DSM 938. The reaction conditions were as described in Example 1 for E. coli and P. vulgaris except the compounds of formula (IIb) and (IIc) were present at a substrate concentration of 0.1 g/l. Where a preparation of DMSO reductase was used, the reaction conditions were as follows:

Cells of R. capsulatus DSM 938 were grown phototrophically in 25 l carboys containing RCV medium supplemented with 50 mM DMSO between two banks of 100 W tungsten bulbs. Cells were harvested by cross-flow filtration followed by centrifugation at 10,000 rpm in a Beckman GSA rotor for 30 min. The pellets were washed once with 50 mM Tris/HCl pH 8.0 and resuspended to approximately 2 g/ml in 50 mM Tris/HCl pH 8.0 + 0.5 M sucrose and 1.5 mM EDTA (STE buffer). Lysozyme was added at 0.6 mg/ml and the suspension (approx 1l) was stirred at 30°C for 15 min. After centrifugation as described above the supernatant (periplasmic fraction) was decanted off and brought to 50% saturation with ammonium sulphate. Following centrifugation the supernatant was brought to 70% saturation and re-centifuged. The 50 - 70% pellet was resuspended in a minimum volume of 50 mM Tris/HCl pH 8.0 and dialysed against 3 x 100 volumes of the same buffer being concentrated to approx 5 ml by ultra-filtration through an Amicon PM 10 membrane. The concentrated sample was brought to 150 mM NaCl, charged onto a Sephacryl S200 gel filtration column (column vol = 510 ml) and eluted with 700 ml of 50 mM Tris/HCl pH 8.0 + 150 mM NaCl. Peak enzyme activity eluted at approx 320 ml (data not shown) and peak fractions were pooled and concentrated as above.

The "RCV" medium used in this Example had the following composition (g per litre of deionised water or as mM):

Propionate	30 mM

$(NH_4)_2SO_4$	1 g
KPO_4 buffer	10 mM
$MgSO_4.7H_2O$	120 mg
$CaCl_2.2H_2O$	75 mg
Sodium EDTA	20 mg
$FeSO_4.7H_2O$	24 mg
Thiamine hydrochloride	1 mg
Trace element soln.	1 ml

Trace element solution (per 250 ml)

H_3BO_3	0.7 g
$MnSO_4.H_2O$	398 mg
$Na_2MoO_4.2H_2O$	188 mg
$ZnSO_4.7H_2O$	60 mg
$Cu(NO_3)_2.3H_2O$	10 mg

The purified DMSO reductase from Rhodobacter capsulatus DSM 938 was used in the photo-catalysed assay for the reduction of sulfoxide. A 5 ml gas-tight syringe was filled with approx 3 ml of degassed assay buffer, 50 mM Tris/HCl, 10 mM EDTA, pH 8.0. Flavin mononucleotide (FMN) and sulfoxide were added to the syringe to give final concentrations of 250 µM and 0.3 mM respectively (based on a final volume of 5 ml). The FMN was then reduced via illumination with a tungsten lamp. The DMSO reductase sample (2-10 mg : 2-10 µM) was then added to the solution and the volume made up to 5 ml with degassed buffer. The syringe was then illuminated as before. A 1 ml sample was removed immediately (t=o) and subsequent sampling repeated over the desired timecourse.

Detection of Products

The bioreduction of the compounds of formula (IIb) and (IIc) was followed by reverse phase HPLC as described in Example 1 except that the retention times were as follows:

TABLE 4

Compound of Formula	Retention Time (min)
Ib	8.1
IIb	4.2
Ic	10.5
IIc	5.7

The enantiomeric composition of the compounds of formula (IIb) and (IIc) remaining was investigated by the method of Example 1 except that in the chiral HPLC step, the solvent composition, flow rate and retention time were as follows:

TABLE 5

Compound of Formula	Solvent Composition	Flow Rate (ml/min)	Retention Time (min)
IIb	Hexane/Ethanol (70:30% v/v)	1.0	32.3 (Enantiomer A) 36.6 (Enantiomer B)
IIc	Hexane/Ethanol (70:30% v/v)	0.5	34.3 (Enantiomer A) 36.3 (Enantiomer B)

The enantiomer which eluted first is referred to as enantiomer A and the second as enantiomer B.

The following results were obtained:

TABLE 6

Biocatalyst	Time (hr)	Ratio of enantiomers of formula (IIb)		Conversion (%)
		A	B	
E coli ATCC 33694	0	50	50	-
	2	45	55	68
	20	40	60	-
	44	<1	>99	89
P vulgaris NCIMB 67	0	50	50	-
	2	53	47	75
	6	53	47	75
	48	53	47	75

Conversion values were for the percentage conversion of the compound of formula (IIb) to the compound of formula (Ib).

TABLE 7

Biocatalyst	Time (hr)	Ratio of enantiomers of formula (IIc)		Conversion (%)
		A	B	
E. Coli ATCC 33694	0	50	50	-
	2	39	61	83
	20	21	79	96
P. vulgaris NCIMB 67	0	50	50	-
	2	40	60	45
	6	40	60	54
	48	38	62	85
DMSO reductase (R. capsulatus DSM 938)	0	50	50	-
	0.25	41	59	31
	1.5	41	59	54

Conversion values were for the percentage conversion of the compound of formula (IIc) to compound of formula (Ic).

EXAMPLE 3

The reductive resolution of compounds of formula (IId) and (IIe) was investigated.

E. coli ATCC 33694 and Proteus mirabilis NCIMB 8268 were screened for the reductive resolution of compounds of formula (IId) and (IIe). Both organisms were grown anaerobically with fumarate as terminal electron acceptor and glycerol (E. coli) or glucose (Proteus mirabilis) as carbon source according to the method of Example 1. After 48 hrs growth at 35°C the cells were harvested by centrifuging at 8k rpm and 4°C and washed by resuspending in 10 mM sodium phosphate buffer, pH 7.6 and centrifuging as above. The cells were finally resuspended in 100 mM sodium phosphate buffer, pH 7.6. The dry cell weights were 8 g/l (E. coli) and 4.5 g/l (Proteus mirabilis) for the compound of formula (IId) and 4.3 g/l (E. coli) and 3.9 g/l (Proteus mirabilis) for the compound of formula (IIe). The reductive resolution of the compounds of formula (IId) and (IIe) was investigated in an autotitrator containing 40 ml cell suspension, 100 ppm of either substrate and 1 % w/v glucose as energy source.

Detection of Products

The bioreduction of the compounds of formula (IId) and (IIe) was followed by reverse phase HPLC as described in Example 1 except that the retention times were as follows:

TABLE 8

Compound of formula	Retention Time (min)
Id	13.7
IId	5.0
Ie	9.4
IIe	4.3

The enantiomeric composition of the compounds of formula (IId) and (IIe) remaining was investigated by the method of Example 1 except that in the chiral HPLC step, the solvent composition, flow rate and retention time were as follows:

TABLE 9

Compound of Formula	Solvent Composition	Flow Rate (ml/min)	Retention Time (min)
IId	Hexane/Ethanol (70:30 % v/v)	1.0	12.9 (Enantiomer A) 21.7 (Enantiomer B)
	Hexane/Ethanol/Methanol (40:55:5 % v/v)	1.0	7.4 (Enantiomer A) 10.6 (Enantiomer B)
IIe	Hexane/Ethanol (70:30 % v/v)	1.0	26.0 (Enantiomer A) 30.5 (Enantiomer B)

The enantiomer which eluted first is referred to as enantiomer A and the second as enantiomer B.

The results for the reduction of the compound of formula (IId) by both E. coli ATCC 33694 and P. mirabilis NCIMB 8268 were as follows:

TABLE 10

Microorganism	Conversion (%)	Enantiomeric excess (%)	Enantiomer	E
E. Coli ATCC 33694	79	88	B	4
P. mirabilis NCIMB 8268	66	90	B	8

The conversion values were for the percentage conversion of compound of formula (IId) to compound of formula (Id).

The reduction of the compound of formula (IIe) by E. coli and P. mirabilis afforded 'B' enantiomer in high enantiomeric excess.

TABLE 11

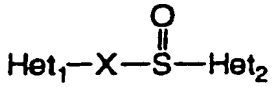
Microorganism	Conversion (%)	Enantiomeric excess (%)	Enantiomer	E
E. Coli ATCC 33694	78	98.2	B	7
P. mirabilis NCIMB 8268	70	>99	B	11

The conversion values were for the percentage conversion of the compound of formula (IIe) to the compound of formula (Ie).

These conversion figures were determined from the aqueous dissolved concentration of sulfoxide at the end of the reaction. The magnitude of the enantiospecificity constant is similar to that obtained for the reductive resolution of the compound of formula (IIa) by E. coli ATCC 33694 and Proteus mirabilis NCIMB 8268.

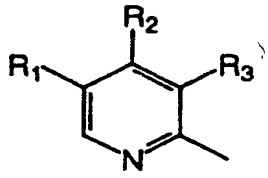
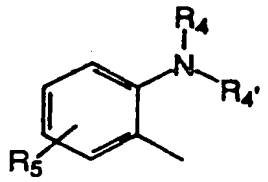
CLAIMS

1. A method of obtaining a compound of formula (II) either as a single enantiomer or in an enantiomerically enriched form:

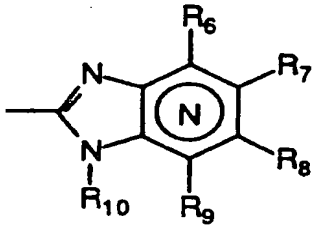
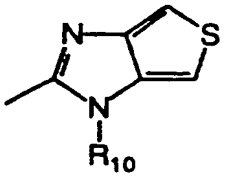


(II)

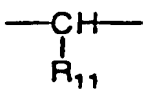
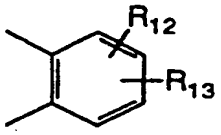
wherein:

Het $_1$ is  or 

and

Het $_2$ is  or 

and

X is  or 

wherein:

N in the benzimidazole moiety of Het_2 means that one of the carbon atoms substituted by any one of R_6 to R_9 optionally may be exchanged for an unsubstituted nitrogen atom;

R_1, R_2 and R_3 are the same or different and selected from hydrogen, alkyl, alkoxy optionally substituted by fluorine, alkylthio, alkoxyalkoxy, dialkylamino, piperidino, morpholino, halogen, phenylalkyl, phenylakoxy;

R_4 and R_4. are the same or different and selected from hydrogen, alkyl, aralkyl;

R_5 is hydrogen, halogen, trifluoromethyl, alkyl, alkoxy;

R_6 - R_9 are the same or different and selected from hydrogen, alkyl, alkoxy, halogen, haloalkoxy, alkylcarbonyl, alkoxycarbonyl, oxazolyl, trifluoroalkyl or adjacent groups R_6 - R_9 may complete together with the carbon atoms to which they are attached optionally substituted ring structures;

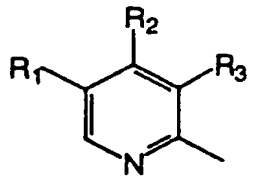
R_{10} is hydrogen or alkoxycarbonyloxymethyl;

R_{11} is hydrogen or forms an alkylene chain together with R_3;

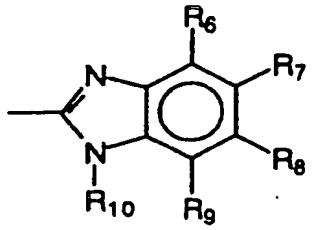
R_{12} and R_{13} are the same or different and selected from hydrogen, halogen or alkyl;

which method comprises stereoselective bioreduction of a compound of the formula (II) in racemic form.

2. A method according to claim 1 wherein:

Het $_1$ is 

and

Het $_2$ is 

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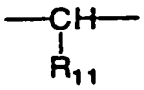
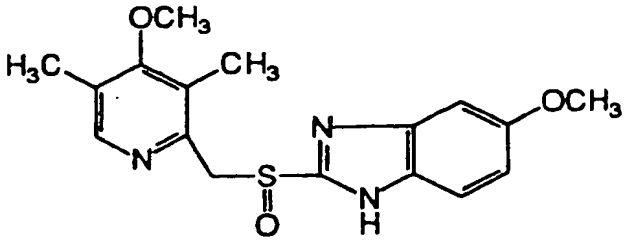
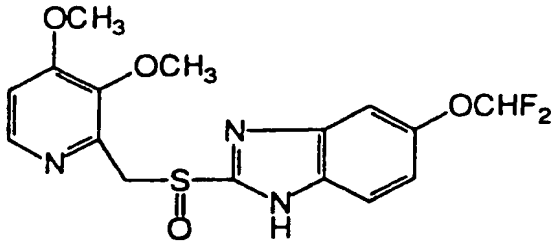
X is 

(wherein R_1, R_2, R_3, R_6 - R_9, R_{10} and R_{11} are as defined in claim 1).

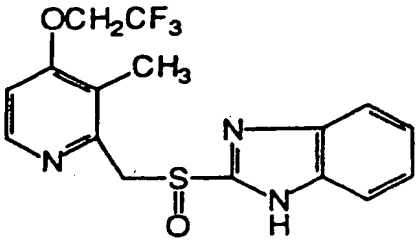
3. A method according to claim 1 or 2 wherein the compound of formula (II) is a compound of formula:



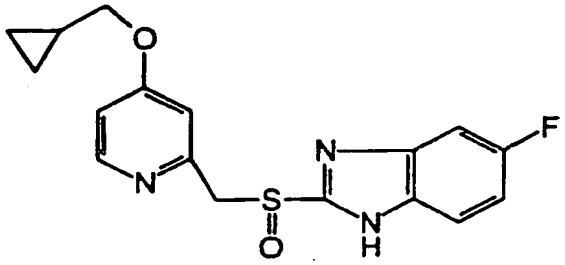
(IIa)



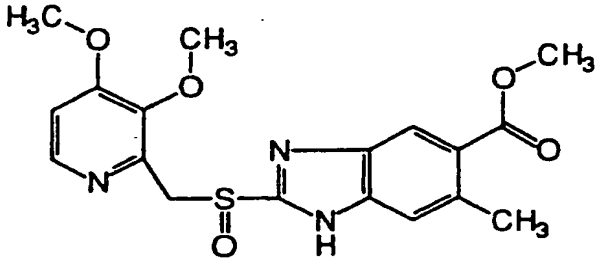
(IIb)



(IIc)



(IId)



(IIe)

4. A method according to any one of the previous claims wherein a single enantiomer of the compound of formula (II) is obtained.

5. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIa) and the bioreduction is carried out with

Proteus vulgaris
Proteus mirabilis
Escherichia coli
Rhodobacter capsulatus or
the DMSO reductase isolated from R. capsulatus.

6. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIb) and the bioreduction is carried out with

Escherichia coli.

7. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IIc) and the bioreduction is carried out with

Proteus vulgaris
Escherichia coli
Rhodobacter capsulatus or
DMSO reductase isolated from R. capsulatus.

8. A method according to claim 3 wherein the compound of formula (II) is the compound of formula (IId) or (IIe) and the bioreduction is carried out with

Proteus mirabilis or
Escherichia coli.

9. A method according to claim 1 substantially as described in any one of the Examples.

10. A compound of formula II as defined in claim 1 as a single enantiomer or in an enantiomerically enriched form obtained by the method claimed in any one of claims 1 to 9.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 95/01416

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12P 11/00, C07D 401/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0005129 A1 (AB HÄSSLE), 31 October 1979 (31.10.79) --	1-4
A	Biosci. Biotech. Biochem., Volume 58, No 3, 1994, M. Abo et al., "Enantioselective Reduction of the Sulfoxideto Sulfide in Methyl Phenyl Sulfoxide by Dimethyl Sulfoxide Reductase from Rhodobacter sphaeroides f.s. denitrificans" page 596 - page 597 --	1-10
A	Biochemical Pharmacology, Volume 48, No 2, 1994, E. Kashiyama et al., "Chiral Inversion of Drug: Role of Intestinal Bacteria in the Stereoselective Sulphoxide Reduction of Flosequinan" page 237 - page 243 --	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

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International application No.
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